THE ETIOLOGICAL RELATION OF BACILLUS ACTINOIDES TO BRONCHOPNEUMONIA IN CALVES.

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PLATES 42 TO 50.

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In the early months of 1917 an outbreak of pneumonia occurred among calves belonging to a large dairy herd in which about 100 calves are raised annually. Up to May 1, eleven cases had been autopsied. Nine of these were killed in advanced stages of the disease and two died. Scattering cases occurred at the rate of about one a month into November. Of these, one died and seven were killed. In the course of the investigations a minute bacillus was isolated whose etiological relation to the pneumonia was left in doubt.[1] In this outbreak a peculiar disease of the kidneys occurred in ten out of the nineteen cases. The lesions were situated in the cortex and consisted of foci of sclerosis with destruction of the secreting tissue.

More than 2 years later, in October, 1919, there began in the young stock of the same herd a similar series of cases. Up to March of 1920, ten cases came to autopsy. Of these four had died and six were killed. The kidney lesions were absent. A belated case was observed in June. There may have been more cases which recovered without being detected, since no careful clinical examination was made and the disease was recognized only when the respiration had become sufficiently abnormal to warrant removal from the herd. The ratio of animals openly diseased to the entire number of calves was relatively low. This may have been due to the fact that the calves were kept in groups of six to eight in adjoining open pens, those of nearly the same age being kept together.

[1] Smith, T., *J. Exp. Med.*, 1918, xxviii, 333

In the second outbreak the lesions of the respiratory tract, more particularly of the lungs, were the chief if not the only ones. In the first, as stated above, a nephritis was associated with over half the cases. In the present article references to the nephritis will be omitted and made the subject of a separate paper. A description of the bacillus was published in 1918.[1] Its chief distinguishing characters are briefly as follows

1. Multiplication in the form of small flakes up to 1 mm. in diameter which consist of parallel bundles of filaments, each filament terminating in two club-like expansions, one at each end. The flake thereby becomes more or less rounded, mulberry-like, and resembles a sphere with clubs projecting from the central mass. In this *Actinomyces*-like growth and within the sheathing filaments are chains of minute bacilli.

2. The sheaths and clubs are produced in the condensation water of coagulated serum and to a less extent on the sloped surface but not on agar plus tissue or blood. On these latter media the growth is feeble and the organism appears in the form of rods without the sheaths. It often fails entirely.

3. On ordinary media such as plain agar and bouillon the bacillus multiplies very faintly or not at all.

4. After 10 or more days growth on the agar plus blood or tissue, minute refringent bodies are found free in large numbers and also within the remaining rods. In some cultures all bacilli had disappeared. This phenomenon has been tentatively interpreted as spore formation.

5. The bacillus fails to produce any appreciable lesions in small animals.

Pathological Anatomy and Bacteriology of Individual Cases.

The gross appearances of the lungs differed very much from animal to animal but the cases could be grouped according to certain characters into acute, chronic, and intermediate types. The important features of the pneumonia, both pathological and bacteriological, are best presented by a brief description of certain cases which will form the basis for a discussion of the etiology. For the names applied to the different lobes of the cow's lungs in these pages, the reader is referred to the description of Text-figs. 1 and 2, page 450.

No. 440.—Female calf, aged 49 days. Died suddenly Nov. 12, 1919, and was autopsied within a few hours. There is no history of any pronounced illness preceding death, although this may have been overlooked by the attendant.

The lesions are restricted to the respiratory organs. The lungs are affected symmetrically. Both cephalic and ventral lobes are solidified with the exception

of six or more small air-containing regions along caudal border of right cephalic lobe and several along dorsal margin of the same lobe. Near root of right ventral lobe there is an air-containing region several centimeters in diameter. The large caudal lobes are solidified in the cephalic third or half. The involved tissue covers much more of the diaphragmatic than of the dorsal surface of these lobes. Air-containing lobules encroach on the hepatized territories and foci of collapsed lobules extend into and permeate the air-containing portions. The small azygous lobe consists of inflated and dark red collapsed regions intermingled.

The involved lobes are as firm as liver tissue to the touch. Their dimensions are about twice those of lungs normally collapsed. The gross appearance of the pneumonic regions differs from lobe to lobe. The cephalic and ventral lobes show closely set, grayish dots 1 to 3 mm. in diameter on a dark red ground, excepting a strip comprising the dorsal border, where the tissue is uniformly dark red, lacking the lighter dots. Similarly, the caudal lobes show the mottled character in a strip adjacent to the ventrals and the smooth, more uniform condition caudally, bordering on the still air-containing regions of these lobes. The mottling is also found on section. Embedded in one ventral lobe there are several foci, 4 to 5 mm. in diameter, which are made up of a semisolid caseous matter. Quite small foci 1 to 2 mm. in diameter of similar character are found here and there in the other lobes.

Larynx and trachea are normal. At bifurcation there is a mass of whitish mucoid substance. Both bronchi are deeply congested and flecks of whitish, soft, mucoid material are scattered over the mucosa. When the lobes are cut across and gently compressed, minute whitish molds are forced out of the smaller bronchioles. The lymph nodes, both mediastinal and bronchial, are somewhat enlarged, on section slightly congested and juicy. Occasional whitish, point-like foci are seen on the cut surface. The pleura is normal. The heart is flabby; pericardium normal. In the fat around the base of the right ventricle there is some patchy hemorrhage. Both sides of the heart contain large, dark, soft clots extending as cylindrical molds into all the large vessels.

Sections from fixed and hardened tissue of the various lobes stained in eosin and methylene blue present the following features. The small air tubes are filled with a mixture of cells and a peculiar, faintly bluish red, homogeneous material, probably mucus. The relative amount of this and of cellular material varies from tube to tube. Within the parenchyma there are foci consisting each of a group of alveoli which are packed with cell masses and the homogeneous substance referred to above. Among the desquamated alveolar epithelial cells are smaller cells the nuclei of which have contracted into irregular star-shaped, solidly stained masses of chromatin or into other irregular shapes, simulating the nuclei of polynuclear leucocytes. They are either lymphocytes or endothelial cells. A few polynuclear leucocytes permeate the mass of cells and debris. The cell foci may be so numerous as to coalesce, or there may be between them a zone of alveoli in which only a few desquamated alveolar cells are seen. It is these cell foci which appear to the naked eye as the grayish subpleural dots. The cell masses in the bronchioles and the alveoli are the same in character.

Another feature of significance is the presence within the smallest air tubes of what appear to be ingrowths from the wall (Figs. 1 to 3). These ingrowths consist of cells of the types described as present in the alveoli, embedded in a feebly stained matrix. The epithelium is missing where the ingrowth takes place. Within this matrix are dense masses of bodies varying in shape from minute rods in small colonies to groups of roundish bodies 2 microns in diameter with only the outline stained (Figs. 4 to 7). These latter bodies are also found in the alveolar cell masses but much less abundantly than colonies of minute bacilli (Figs. 8 and 9).

The origin of the cloud-like masses of bacteria pushing into the lumen of the air tube is obscure. A prolonged examination of sections from this and other cases makes it probable that the bacteria start in the cytoplasm of the epithelial cells, develop into colonies, and thereby cause a great increase in the size of the cells and their eventual destruction. The mass thus projects into the lumen where some of the migrating cells fuse with it, producing the characteristic obstructing plug. It is doubtful whether there is any actual hyperplasia of the epithelial cells. In the cell foci of the parenchyma a similar invasion of the alveolar cells takes place, leading to destruction of such cells and the appearance of hemorrhages and of the lymphocyte cell masses.

Cultures were made on slanted coagulated horse serum and slanted agar with and without a few drops of horse blood. All tubes were hermetically sealed and incubated. The cultures were made both by transferring bits of lung tissue and by inoculating the tubes with a heavy platinum wire thrust into the pneumonic tissue. After several days, growth appeared and in all but a few tubes *B. actinoides* was present in pure culture. In the remaining tubes a few foreign colonies were also present.

No. 450.—Black and white female calf. Born Oct. 27, 1919. Attendant reported that on Nov. 27 the calf did not eat its food and that breathing was rapid. From that day on the temperature was taken several times and found to be around 40.5°C. There was much coughing, the respirations continued short and superficial, and emaciation progressive. Dec. 4. The calf, then weighing 85 pounds, was killed by stunning with a heavy blow, clamping the trachea to prevent aspiration of stomach contents into the lungs, and opening the vessels of the neck. There was nothing noteworthy about the organs excepting the respiratory tract.

When the thorax was opened the lungs collapsed with force. The distribution of the pneumonic lesions is somewhat less extensive than in the preceding case. Both cephalics and ventrals are entirely consolidated. The cephalic half of the azygous lobe and a narrow strip along the cephalic margin of the right caudal lobe are involved. The left caudal lobe is intact. There is a narrow margin of air-containing lung tissue along the dorsal ridge of both lungs almost to the cephalic tip, also small inflated lobules in the left ventrocephalic lobe.

The condition of the consolidated lobes is not the same throughout. The right cephalic and the right ventral lobes are much larger than the normal collapsed

condition would be. They are grayish red to grayish yellow in appearance. The remainder of the affected lung is but a trifle larger than in the normal collapsed state and dark red with a faint, regular mottling of a lighter color. In this lung the grayish dots or granulations characteristic of the preceding case are absent. The pleura is normal.

The lower portion of the trachea and the main bronchi at the bifurcation contain masses of a viscid, opaque, whitish, dough-like mucopus. The same glairy, pearly white masses can be expressed from the cut ends of the minute air tubes of the dark red lobes. Only a small amount is expressible from the air tubes of the large grayish lobes. This glairy matter is very tenacious and breaks up into fragments when crushed between cover-glasses. The bulk of the material is made up of polynuclear leucocytes. The dark reddish tissue is quite moist when rubbed on covers, the grayish less so. The latter leaves on the cover-slip a smeary, milky film. Projecting from the right ventral lobe is a whitish mass the size of a pea. It is made up of a thin walled cyst filled with a readily dislodged, dough-like mass. Cover-glass films show abundant alveolar epithelium, polynuclear leucocytes, and several dense masses of very fine, rather feebly stained bacilli. Films from tracheal and bronchial mucopus and from lung parenchyma contain the same cellular and bacterial elements.

The difference between the gross pictures of the lungs of Nos. 440 and 450 is paralleled by differences in the microscopic picture. The peculiar cell infiltration in No. 440 has disappeared and polynuclear leucocytes have taken its place. The cell plugs in the air tubes contain chiefly polynuclears among which are scattering alveolar cells. The peculiar amorphous, homogeneous material which was regularly associated with colonies of bacteria in No. 440 is absent and bacteria are not detected. The parenchyma is the seat of polynuclear infiltration in foci which coalesce in the older stages and in the more recent stages gather irregularly in the collapsed tissue. The invasion of new territories is characterized by a ring of fibrin-blocked alveoli around the invaded air tube and occasionally hemorrhages. The interlobular tissue contains oval masses of fibrin lodged in the distended lymph spaces.

The difference between the two cases is further indicated by a new feature. All small air tubes are surrounded by a loose zone of cells which resemble plasma cells in form and the basophilic character of the cytoplasm and which collect in the subepithelial tissue.

Cultures prepared from the different lobes by transferring bits of lung tissue to horse serum, agar and blood, and plain agar, as well as by inoculating the same kind of media with heavy and fine platinum wire forced into the lung tissue yielded cultures of *B. actinoides* which were pure with the exception of those from the azygous lobe in which a few large streptothrix-like colonies appeared. The tubes inoculated with the heavy platinum wire developed, whereas most of those inoculated with a fine wire remained sterile.

No. 446.—This is a case which duplicates in all particulars No. 450 (Fig. 14). This calf was born in September and was 2 months and 2 days old when killed.

Cultures prepared from the different lobes in the manner described for No. 440 developed, with few exceptions, growths of *B. actinoides*. A few stray colonies of other kinds were present in several tubes.

No. 432.—This case may be classed as intermediate between the first and the following group. Female, born June 21; killed Oct. 21, when 4 months old. The meager data indicate that it was sick with some respiratory affection late in July and early in August. Early in October the respiration became short and quick, there was frequent coughing, and the calf breathed with mouth open. The respirations continued short and labored until it was killed. The temperature fluctuated between 39° and 40°C. The animal was killed by a stunning blow on the head, clamping trachea, and severing vessels of neck.

The consolidation of the lungs is of the usual extent. The pneumonic process involving completely the cephalic half of both lungs has invaded both caudal lobes and is separated from the air-containing tissue by a sharp, irregular, jagged line. Pneumonic lobules also appear as islands in these lobes. The trachea contains whitish, mucoid masses and the small air tubes of the affected lobes are filled with molds of similar material.

The hepatization is very firm, the affected lobes about twice the dimensions of the normal collapsed state. The surface is mottled like that in No. 440. Necrosis is absent. The cut surface shows the walls of the small air tubes distinctly thickened. In general the gross appearance indicates that the process is more or less of the same age throughout, except in the caudal lobes, where it is freshest. The mucopurulent molds from the small air tubes and the loose masses in the trachea contain large numbers of very minute and somewhat larger bacilli. Histological examination of sections from the different lobes indicates a transition in the pathological process from that of No. 440 to that of No. 450. In some regions the infiltrating cell masses are mononuclear, the contents of the minute air tubes largely made up of the homogeneous, bluish stained substance and groups of minute bacilli. In others, polynuclears predominate both in the parenchyma and the air tubes, and bacteria are rare or absent.

The bacteriology of the processes in this lung is complicated. On the agar and blood agar slants many roundish, rather fleshy colonies develop which have the cultural characters of the hemorrhagic septicemia group but with very low virulence. The general presence of *B. pyogenes* is indicated by liquefying colonies in all horse serum tubes. A third type of bacilli not identified was present in some tubes. Identifiable by its peculiar growth, *B. actinoides* was demonstrated as occurring in most tubes, but repeated attempts to obtain it in pure culture failed, owing to the predominance of the other types.

A type of pneumonia more acute than that represented by No. 440 was observed in three cases. No. 429, the first of the present outbreak to come to autopsy, was born September 7 and died October 12. The second, No. 436, was born September 20 and died

November 4. The third, No. 447, was born October 17 and died November 29. The three calves were thus respectively 35, 45, and 43 days old at the time of death. The three cases present certain differences among themselves.

No. 429.—All lobes are much enlarged over the normal collapsed size. There is an adhesion of most of cephalic and ventral lobes to ribs and to pericardium. The attachments are easily broken except the adhesion of right cephalic lobe to pericardium which is not separable. Consolidation of all but one-half of right caudal and two-thirds of left caudal lobe. There is a general faint putrefactive odor emanating from the lungs. The affected lung tissue is dark red and sprinkled over with numerous yellow, cheesy foci 2 to 5 mm. in diameter and projecting slightly above the pleura. These foci permeate the lung tissue with the exception of the caudal and azygous lobes in which such foci are few and small. Besides the small foci the right cephalic lobe contains a sequestrum 2 by 2 by 5 cm., yellowish, and firm like rubber. The trachea is uniformly reddened and covered with whitish, viscid flakes.

No. 436.—Extent of lung involvement and size of affected lobes are as in No. 429. The lung tissue resembles that of No. 440. It is sprinkled over densely with lighter grayish foci about 0.5 mm. in diameter. On pressure very little fluid and only a few consistent molds can be expressed from the air tubes. The multiple necroses found in No. 429 are absent. The mucosa of the trachea is uniformly congested, the bronchi deeply so, bordering on hemorrhage. The mucosa has on it some very thin patches of a pseudomembrane.

No. 447.—The involvement is somewhat less extensive than in Nos. 429 and 436. The pneumonic lobes are firm, only moderately larger than the normal collapsed state, uniformly dark red, and interspersed with a few inflated lobules. There is no distinct mottling with lighter dots as in No. 436, but the surface is rather variegated with larger patches of lighter color. Necrotic foci are not found. The trachea is clean and normal to the bifurcation where some flour-paste-like masses of mucopus are lodged. The main bronchi of affected lobes are filled with a similar thick pasty mass but those of the caudal lobes are free.

The microscopic picture in these three cases as constructed from sections of the different lobes is very much like that of No. 440 already described. The consolidation is due to focal infiltration of the parenchyma with the type of cell described under No. 440. Polynuclears are scarce or absent. The minute air tubes contain, besides cell masses, the homogeneous, bluish stained material, and it is in and among this material that masses of minute bacteria occur. The necroses of No. 429 occupy variable areas of lung tissue in which the alveoli have become impacted with the cell type mentioned. In these cases the gathering of plasma cells in the mucous membrane of the air tubes had not yet begun.

Cultures made from different parts of the lungs of the three cases indicated the presence of various species. Present in all three was *B. pyogenes*, especially abun-

dant in No. 429, less so in No. 436, and least so in No. 447. The sections containing the necrotic foci of No. 429 stained according to Gram-Weigert showed large, deep blue spots resolved as minute bacilli and evidently colonies of *B. pyogenes*. Several other types of colonies were found in the cultures. *B. actinoides* could not be detected in cultures from Nos. 429 and 436. Several cultures from No. 447 contained large numbers of colonies of *B. actinoides* but pure cultures failed because of the presence of *B. pyogenes*. Inasmuch as the type of disease was the same in the three cases, it is probable that *B. pyogenes*, being an acid producer, interfered with the development of *B. actinoides*.

Another group of cases is represented by calves in which the disease processes have gone on more slowly and to a much farther stage. The involved tissues have become necrotic and encapsuled, causing firm adhesions of the pleura to the chest wall and pericardium. There is a broad zone of plasma-like cells around the smaller air tubes, varying in size with the age of the calf.

Case 455.—Female calf born July 26; 4 months old lacking 3 days when killed. The attendant reported that this calf was sick with scours in August. Late in September it had a cold and in November symptoms of pneumonia were present. When killed it was very thin, the respirations short and labored. The cough was frequent, the temperature 40°C.

In this case also the pathological changes were limited to the respiratory organs. When the sternum was removed, the lungs collapsed vigorously. The pneumonic process had the usual extent and symmetrical distribution. The appearance of the lobes was much the same. The tissue is a reddish yellow and beset with closely crowded grayish areas, about 2 mm. in diameter, although varying more or less in size. Similar areas are seen on the cut surface. In the right cephalic lobe the grayish areas are larger and tend to coalesce. In the free tip of this lobe there is a group of larger foci about pea size, made up of thin walled sacs containing what appears to be in part necrotic lung tissue, in part mucus and pus. The pleura covering these foci gives rise to delicate fibers attaching lobe to pericardium. The mucosa of the trachea is normal and has lying in it white, curdy, viscid masses 4 to 5 mm. in diameter made up of polynuclear cells, alveolar epithelium, mucus, and some minute bacilli. All the small air tubes in the consolidated lobes when compressed exude thick, glairy, pearly white masses. Microscopically this material is like that in the lower trachea. All thoracic lymph nodes are distinctly larger than normal; on section quite moist and exuding a milky fluid.

The histological picture differs in degree from that of the more acute types. The most striking character is the presence of a zone of plasma cells under the epithelium of the air tubes, varying in thickness and especially broad in some lobes. The smallest air tubes are distorted, or else nearly obliterated so that their presence is only indicated by the plasma cell groups and zones. These cell zones plus

a broadening of the submucous layer probably represent the grayish areas seen from the surface and on section. The lung tissue around and between these zones is either slightly inflated and contains some desquamated cells, or else is collapsed or filled with polynuclear leucocytes. In sections bacteria are not seen, except in one large mass growing into and nearly obliterating the already dilated and deformed tube. In this mass colonies of fine, rather feebly stained bacilli are present. The flora of the pneumonic lobes of this case resembles somewhat that of No. 432. Of the large number of cultures, about thirty in all prepared with minute and pea size bits of lung tissue, all showed development. The colonies in each tube were few in number. *B. pyogenes* predominated in being present in most tubes. A second form, consisting of 2 to 3 mm., smooth, grayish, translucent colonies which tended to flow down the slant, was probably a variety of *B. bovisepticus*. Several other forms were present. *B. actinoides* was detected in only two cultures among other forms. Among several cultures from bits of tissue from thoracic lymph nodes, one contained *B. pyogenes*.

Case 462 (Text-Figs. 1 and 2 and Fig. 12).—Female, born Nov. 4; killed Jan. 7, weighing at this time 102 pounds. It began life with scours which persisted for some weeks. Evidences of respiratory trouble began to appear Dec. 27. Although only a few days over 2 months old when killed, it presented advanced destructive lesions in larger number than any other case of this small outbreak. The pathological changes, if we except the general disappearance of fat deposits, were restricted to the lungs. Both cephalic and ventral lobes and a small adjacent zone of both caudal lobes were involved. The smaller lobes were adherent to the chest wall and pericardium through thin bands severed with some difficulty. In all but the caudal lobes are isolated or agglomerated, whitish, projecting nodes 5 to 10 mm. in diameter. The lung tissue between them is dark red and beset with paler grayish areas 2 to 3 mm. in diameter. The nodes are sacs containing a glistening, pearly white, viscid, flour-paste-like mass, rather sticky and not easily spread on cover-slips. It consists of cellular elements among which are dense masses of minute bacilli. Within this mucoid, whitish coating is a nucleus consisting of a lobulated mass not so white as the mucoid covering, more brittle, and finely spongy. The gross and microscopic characters indicate necrotic lung tissue. The walls of the cavities containing the sequestra are 1 to 1.5 mm. thick, and smooth. Where a number of cavities are close together they evidently communicate, since pressure continues to force the necrotic and mucoid masses out of all the cavities through one opening. Stained films of tracheal mucopus and pus from the necrotic foci show besides cellular elements many minute bacilli frequently in dense masses. The air tubes of the affected lobes contain the same viscid, mucoid, purulent masses found in preceding cases.

The histological changes found in the various lobes do not differ materially from those of the advanced, chronic type. In general there is much collapse of the parenchyma with marked broadening of the alveolar walls due to infiltration of endothelial cell types. The collapsed tissue is focally occupied by dense infiltrations of polynuclear cells into the alveoli. In some lobes the normally distended

alveoli contain sparse collections of desquamated cells and polynuclears. The air tubes in some lobules have a dense enveloping zone of plasma cells. In others it is very slight. Sections through the necrotic foci show sequestra of lung tissue enclosed in a dense zone of plasma cells and more or less connective tissue stroma. Bacteria colonies are not seen.

TEXT-FIG. 1.

TEXT-FIG. 2.

Diagrams of involved lung tissue, Calf 462.

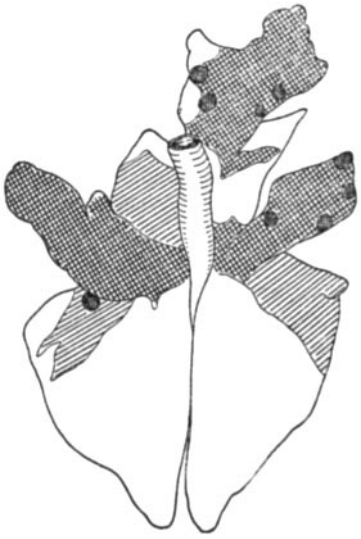
TEXT-FIG. 1. Dorsal aspect.
TEXT-FIG. 2. Ventral or diaphragmatic aspect.

In the text the lobes are designated cephalic, ventral, and caudal. In the right lung the cephalic lobe is relatively very large and has a separate bronchus. In Text-fig. 2 the small median or azygous lobe is shown. The normal lung tissue is unshaded; the heavily shaded circles represent necrotic tissue; the areas cross-hatched designate an older stage than the areas having parallel lines only.

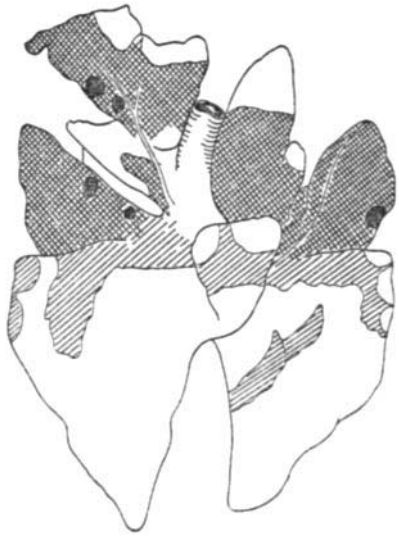
Cultures were prepared from the different lobes on several media as described under Case 440. The extensive necrosis of lung tissue naturally led to the inference that a variety of bacteria would be found. This, however, was not the case. In none of nine horse serum tubes did any liquefaction suggesting *B. pyogenes* occur. In all but one, which remained sterile, *B. actinoides* multiplied in pure culture. Similarly the plain agar tubes containing bits of tissue and blood agar tubes developed pure cultures of *B. actinoides* with two exceptions in which a mold and a fleshy colony appeared.

The following case was the last of this particular outbreak.

No. 508.—Female calf, born May 7. Attendant gives a history of digestive disturbance (scours) beginning a few days after birth and lasting 3 weeks. When first seen June 3, the calf was emaciated, weighing 75 pounds. The respirations were rapid and shallow. Both nostrils were soiled with whitish, mucoid masses. The right ear was held low and was discharging a viscid, pus-like matter. The temperature fluctuated between 39° and 40°C. until June 7, when it was killed.



TEXT-FIG. 3.



TEXT-FIG. 4.

TEXT-FIGS. 3 and 4. Dorsal and ventral aspects of lungs of Calf 508. For explanation of the shading see Text-figs. 1 and 2.

The autopsy showed the usual lung involvement (Text-figs. 3 and 4). Three types of lesions are present: (1) the focal necroses; (2) a firm hepatization with slight enlargement of the cephalic and ventral lobes. which are furthermore permeated with grayish foci 1 to 2 mm. in diameter; and (3) a more recent stage, characterized by a uniformly dark red airless condition. The affected lobes are furthermore variegated by air-containing territories. The lesion referable to the right ear was not traced, owing to the injury inflicted by the blow necessary to stun the animal. A small cavity was found in the temporal bone filled with a puriform liquid which may have been associated with the lesion. Films from this fluid contained numerous polynuclears and several kinds of bacteria (diplococci, bipolar and minute bacilli). Cultures from various regions of the diseased lungs were made. In all a variety of bacteria developed, among them *B. pyogenes*. *B.*

actinoides was detected in all serum cultures. In two tubes the colonies of this bacillus were present in large numbers and among them scattering liquefying colonies of *B. pyogenes*. Pure cultures of *B. actinoides* could not, however, be obtained from these tubes. The sections of material fixed in Zenker's fluid did not show any details differing from those already described.

As an illustration of the condition of the lungs of an animal surviving this type of pneumonia, the following case from the first outbreak is of interest.

No. 6.—This calf was nearly 7 months old when killed. It was born in June, 1916. When received the calf was normal as to temperature, respiration, and pulse. It was thin and hide-bound, but not unusually weak. No early history was obtained. It was killed because unthrifty. The disease was restricted to the right cephalic lobe of the lung. It was adherent to surrounding structures by easily broken fibers. One-half of the lobe is permeated with yellowish white sacs containing a thick creamy fluid. The other (caudal) half of the same lobe presents a grayish mottling of regular pattern. The air tubes of the entire lobe are surrounded by broad, whitish bands, or zones, and contain viscid, glairy molds. The trachea contains a large amount of a viscid, flour-paste-like whitish matter. In sections of the affected lobe the air tubes stand out prominently as thickened tubes. The thickening is due to a broad envelope of plasma cells and new connective tissue around them, while what is left of the parenchyma is slightly emphysematous and contains scattering desquamated alveolar cells (Fig. 13). A number of partly occluding ingrowths into small air tubes are made up largely of an endothelial cell type with an admixture of polynuclears. In a mediastinal lymph node, associated with the diseased lobe, the normal lymphoid cells are almost entirely replaced by the plasma cell type. Cultures from the various lobes developed a rich growth of a bacterium which agreed in cultural characters with the bipolar type of organisms (*B. bovisepticus*). Its virulence towards rabbits was very low. At this time *B. actinoides* was not yet known to the writer. The lesions were ascribed to *B. bovisepticus*.

After an interval of 6 months following the second (1919) outbreak a sporadic case of pneumonia appeared, which is of importance in several directions.

No. 544.—Female calf, born Oct. 6, 1920. It was reported sick by attendant Nov. 18 and transferred to the Institute next day. The animal was emaciated and very weak, unable to stand. The respirations were rapid and shallow. The buttocks were soiled with feces. Temperature on Nov. 19 was between 41° and 41.8°C. during the day. The animal refused all food. Early Nov. 20 the attendant found the calf panting and grunting. It died soon after and was autopsied at 10 a.m.

Besides the extensive pneumonic changes, the only noteworthy lesion is a septic condition of the stumps of both iliac arteries. The lumina are patent and the intima is coated with a thin, grayish, pultaceous layer not removable by washing. One artery contains a grayish yellow, cylindrical thrombus about 2 cm. long. The lungs are extensively hepatized. Even the left caudal lobe is nearly one-half solidified. The cephalic and ventral lobes of both sides have dimensions about twice those of the normal collapsed lung. The hepatization is smooth, grayish red, excepting where the cut bronchi exude whitish, pasty masses. The hepatization of the azygous and the caudal lobes is of a dark red color, less firm. The pleura is free, adhesions absent. Necroses are not detected in any lobe. Both main bronchi contain flakes of thick, glairy matter filling the mouths of many branches. The trachea contains many roundish masses of the same exudate, pea size and coated with froth. Tubes inoculated from various affected lobes, about twenty in all, develop, with one exception, into pure cultures of *B. actinoides*.

The nature of the pathological process was not made clear in the sections examined. The invasion of the epithelium of the ultimate bronchioles shown so clearly in No. 440 could not be seen. The air tubes were plugged with cell masses extending into them from the alveolar ducts. The cells consisted largely of polynuclears, feebly tinted necrotic cells, and cells resembling polynuclears in staining but with roundish nuclei. Throughout the affected lung tissue cells of endothelial and plasma type were abundant and intermingled with the others. Owing to the congested condition of the capillary network their relation to the alveolar walls was not clear. Cloud-like masses of minute bacilli were present among the cell masses and resolved with difficulty into their elements.

To bring this case into relation with those already described it may be assumed that the infecting agent was introduced and disseminated through the lungs in such large numbers and deposited in so many places that the process did not have time to reach the stage of focal necrosis before the calf died.

To the information gained by the two groups of cases in the same large herd may be added some data obtained from the autopsies of calves used for producing small-pox vaccine in the vaccine laboratory of the Massachusetts State Board of Health in Boston. During the year 1908 a small number of pneumonic lungs were found in calves killed after the vaccine had been removed. They weighed between 150 and 200 pounds.

No. A.—Both cephalic lobes are pneumonic. The affected tissue is flesh-red with lighter mottling or else simply collapsed. The air tubes contain molds of creamy pus. Sections show the stage of general polynuclear infiltration of parenchyma and the filling up of minute air tubes with the same kind of cell masses.

In one section there is a cellular irruption into an air tube and in the proliferated mass are colonies of minute bacilli.

No. B.—Both cephalic and ventral lobes are consolidated, and there is also a large focus in the left caudal lobe. Involved tissue is grayish red to grayish yellow, delicately and regularly mottled. The air tubes are distended with whitish, viscid plugs. The process in the focus in the caudal lobe is freshest, that in the free tips of the ventral lobes oldest. In general the histological picture is that of collapse, and infiltration with polynuclears. In one section there are two contiguous foci of cell exudation and hemorrhage undergoing necrosis. Many colonies of fine bacilli are in the periphery of these foci (Fig. 10). These cases, as far as the information goes, suggest an underlying process like that initiated by *B. actinoides*.

The Pathogenic Action of Bacillus actinoides.

The bacteriological study of spontaneous cases pointed to *Bacillus actinoides* as the primary inciting agent of the bronchopneumonia with *Bacillus pyogenes* and more rarely, in older calves, *Bacillus bovisepticus* settling down in the occluded air tubes and necrotic lung tissue. The next step was to determine whether cultures of this bacillus, under suitable conditions, could produce lesions like those found in spontaneous cases. In the following pages is given a brief statement of observations made on inoculated calves, since small mammals had thus far shown themselves quite insusceptible. Some of the calves were inoculated subcutaneously, some intravenously, others into the trachea. The area of the operation was shaved, cleansed with water and alcohol, and painted with tincture of iodine. In the intratracheal injections, a small incision was made through the skin over the trachea before inserting the needle.

No. 467.—Bull calf, born Jan. 21, 1920. Dam had placenta retained at birth and swab from uterus as well as agglutination test was positive for *B. abortus*. Temperature, taken twice daily, fluctuated between 38.5° and 39°C. until Feb. 3, the day of inoculation.

The turbid condensation water of a horse serum culture of *B. actinoides* from Calf 462, 5 days old and under artificial cultivation since Jan. 7, was drawn off, making about 1 cc. in all. This was increased to 3 cc. by the addition of sterile bouillon. The fluid was injected into the subcutis in front of right shoulder. The temperature rose to 39.8°C. within 3 hours and remained at the same level next day. On this day a local swelling appeared over the point of inoculation about 1½ inches in diameter and ¼ to ⅜ inch thick. The temperature fell below 39°C. on Feb. 8. The swelling increased slightly.

On Feb. 19, a second inoculation was made, this time into a jugular vein with the same strain used for the first, subcutaneous inoculation. The condensation water was ground slightly in a crucible to break up the flocculi and calf serum water was added to dilute the suspension. The temperature rose to 40°C. within 3 hours and gradually fell to normal during the night. No further elevation occurred. The calf was killed Mar. 4. The weight had risen from 88 to 125 pounds. The local swelling was about the size of a small hen's egg. The calf was stunned with a heavy blow, a clamp quickly placed on the trachea, and the neck vessels were severed. During this operation the local swelling ruptured and several cubic centimeters of a thick, whitish mass, like flour-paste, were discharged. The autopsy showed normal organs, except for two very small foci of collapse, one in the right cephalic, the other in a caudal lobe of the lungs. The local swelling is adherent to the overlying skin, but readily dissected from the subjacent fascia. The under surface of the capsule is sprinkled with minute hemorrhages. It is 3 to 4 mm. thick, pearly white on section and enclosing a cavity partly filled with a soft, odorless mass like flour-paste. The inner surface of the capsule is dark red to hemorrhagic and delicately mammillated. The cavity has small recesses or pockets. The puriform contents are readily removed from the entire wall. The associated prescapular lymph node is about one-fourth larger than the opposite node and on section smooth and quite juicy. The contents of the abscess consist of cells, only a few of which retain the stain. These are mononuclear. Scrapings from the capsule show a mixture of polynuclear and mononuclear elements and occasional cloud-like masses of minute bacilli. Some of the mononuclear cells contain groups of minute bacilli. *B. actinoides* was recovered from the abscess both on horse serum and on agar plus blood. In one of the latter the growth appeared richer than usual and was found by inoculation into two guinea pigs and by microscopic examination to contain *B. abortus*. The presence of *B. abortus* is accounted for by the infection of the dam which had been demonstrated by suitable tests after the birth of the calf.

No. 336b.—Bull calf, born Feb. 28, 1920. On Mar. 9, it weighed 111½ pounds. A horse serum culture from Calf 462, 5 days old, was used. The condensation water, which contained a dense suspension of flocculi visible to the naked eye and shown to be pure by the microscope, was removed to a sterile tube, 5 cc. of bouillon were added, and the whole was thoroughly shaken. The flocculi were still visible after the shaking. The suspension was injected into a jugular vein at 4 p.m. The temperature rose from 38.8° to 40.6°C. in 5 hours and gradually dropped to 38.9°C. during the night. The calf was kept under observation for 36 days. During this period the temperature remained between 38° and 39°C. and the calf's general condition was normal. On the 36th day it weighed 145 pounds and was sold for slaughter.

No. 479b.—Bull calf, born Feb. 26. Inoculated on Mar. 9, when 12 days old. A first culture from the local lesion of Calf 467 on agar plus blood, 5 days old, was used. The surface growth was transferred with a platinum loop to 5 cc. of bouillon until a fine suspension was produced. This was made up of excessively fine

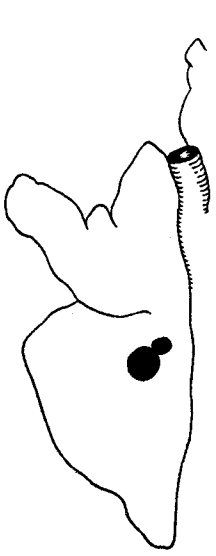
granules when viewed in a strong light. It was injected into a jugular vein. Calf weighed at this time 97½ pounds. The temperature rose from 38.7° to 40°C. within 5 hours. It was only a few tenths above normal next day. The calf continued normal in condition. The temperature remained normal.

On Mar. 24, 15 days after the intravenous injection, a second inoculation was made, this time into the trachea. The condensation water of three horse serum cultures of *B. actinoides*, 4 days old, from Calf 467 was removed to a sterile tube and enough bouillon added to make 12 cc. in all. 5 cc. of this were injected. Within 24 hours a swelling appeared over the trachea, of undefined borders, probably 6 inches long and 2 inches broad. After several days this swelling was somewhat reduced and was now found to be made up of two indurations, one over the point of injection, the other lower down at root of neck. Apr. 5. The right leg was shown to have restricted motion and to be painful when handled. Apr. 15. The right shoulder appeared a trifle larger than the left and tender. The lameness increased. The calf, showing no general symptoms, was killed Apr. 20, 27 days after the intratracheal injection. The only lesions found were the local swellings. One about 2 inches in diameter is situated just caudad of the larynx; a second, 4 inches in diameter, just caudad of the first. Both are firm, tense, slightly fluctuating. They are covered by muscles of the neck and attached to the cervical portion of the thymus. Both are sacs with walls 5 to 10 mm. thick, smooth interiorly, and filled with a soft odorless mass, resembling cottage cheese. They communicate with each other through a narrow opening. The contents consist of lymphocytes and polynuclears, the former greatly predominating. Both kinds of cells largely refuse the stain. There are also filamentous masses, probably necrotic fibers. In the larger abscess a strip of necrotic tissue was found in the caseous contents about 4 inches long, representing the remnants of a vessel about 2 mm. internal diameter. After washing away the pus the external surface was found covered with small papilla-like projections.

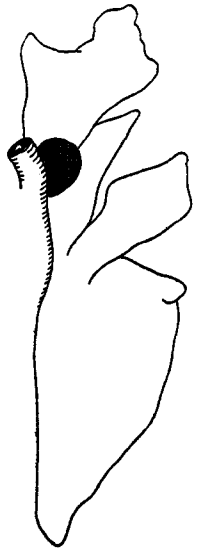
On the prominence of the right shoulder joint there is a swelling about the size of an egg, easily dented by pressure. The tumor is situated between the tendinous ends of the muscles inserted in the scapula. There is marked thickening of the intermuscular fascia radiating from the capsule of the tumor to a depth of several inches. On incision of the thick capsule, an irregular cavity is exposed, with contents mucoid, whitish, quite unlike those of the tumors over the trachea. However, films show the same cell elements, the difference being due to the advanced necrosis of the contents of the other abscesses.

Sections of the abscess walls show a mass of muscular and connective tissue with marked hyperplasia of the connective tissue and some fibrin. There is also a general infiltration with cells of endothelial character. The pus attached to the inner wall consists wholly of cells of endothelial type. Some of these are filled with minute bacilli. There are also clouds of free bacilli feebly stained and easily overlooked. Cultures prepared from both abscesses over the trachea develop into pure growths of *B. actinoides*. No cultures were made from the abscess on the shoulder. The respiratory tract as well as the remaining viscera was normal.

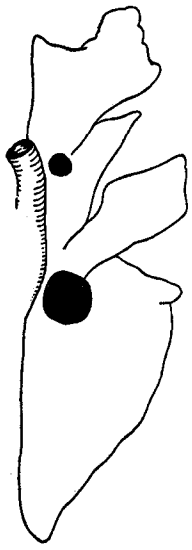
No. 495b.—Female calf, born Apr. 15, 1920. On May 11, 10.30 a.m., when 26 days old and 88 pounds in weight, it was inoculated into the trachea. The condensation water of six horse serum cultures from Calf 479b, 5 days old, was drawn into a fresh tube and 6 cc. of bouillon were added, making 13 cc. of heavily clouded fluid. 8 cc. of this were injected. Animal coughed occasionally after



TEXT-FIG. 5.



TEXT-FIG. 6.



TEXT-FIG. 7.

Effect of the intratracheal injection of cultures of *B. actinoides*.

TEXT-FIG. 5. Necrotic focus in left caudal lobe of Calf 520.

TEXT-FIG. 6. Similar focus in right cephalic lobe of Calf 474b.

TEXT-FIG. 7. Two necrotic foci, one in cephalic, the other near root of right ventral lobe of Calf 495b.

the operation. The temperature rose about 1°C. during the day and was down again the next day. 3 days later the temperature reached 40°C. and fluctuated between 40.2° and 40.7°C. for 6 days, then dropped to 38.3° to 38.7°C. The calf weighed 99 pounds on May 27, having gained 11 pounds in 16 days. It was killed on this day.

The digestive tract is normal throughout. In the respiratory tract there is a bit of semitranslucent mucus on one vocal cord. In the lower trachea there is a similar mass, pea size, made up of alveolar epithelium and polynuclear leucocytes. There are two groups of focal necroses in the lungs (Text-fig. 7). On the dorsal ridge of the right caudal lobe, near root of the right ventral, there

is a small, whitish, opaque, subpleural mass. On palpation, other firm nodules are felt embedded, about half a dozen in all. On section the contents show as thick, cheesy cores in dense capsules. The tissue between them is still air-containing. There is no fresh pneumonia or collapse around them. A similar cheesy nodule is in the right cephalic lobe, embedded in emphysematous tissue. Besides these two necrotic foci there are several collapsed lobules in different lobes. Lymph nodes of thorax are normal. There are 10 cc. of clear fluid in pericardial sac. Both kidneys are spotted everywhere with whitish areas, discrete and confluent, occupying fully one-half of the total cortex. These spots are the bases of cones of fan-shaped outline on section, extending to the medulla. The substance of these foci is glistening, smooth, almost like cartilage in appearance. Urine taken from the bladder soon after death is very pale, clear, faintly alkaline, specific gravity 1,004. Boiling after adding 1 to 2 drops of acetic acid yields a very faint cloud. The liver is slightly fatty.

Sections of the necrotic foci in the lungs show centrally a nucleus of dead lung tissue in which the alveolar structure is still preserved (Figs. 15 and 16). These nuclei are 1.5 to 2 mm. in diameter. In the central core the alveoli are nearly empty. In the peripheral zone, they are filled with a fine granular material in which many cells are embedded. Immediately surrounding the necrotic center is a broad zone in which the lung structure is occasionally seen, but is chiefly replaced by cellular debris embedded in a granular matrix. Dense groups of minute bacilli are in the outer layers of this zone where many alveolar cells are still recognizable. Outside this zone is a layer of fibroblasts and newly formed capillaries. The whole is enclosed in a mantle of lymphoid cells, occupying the meshes of the compressed lung tissue. In the vicinity of the necrotic focus there are zones of lymphocytes around the ultimate and next larger bronchioles, associated with collapse and broadening of alveolar walls through cell infiltration. In some air tubes small groups of polynuclears are seen. Sections of the kidney lesions show interstitial hyperplasia with shrinkage and disappearance of glomerular tufts, dilatation of some convoluted tubules and disappearance of others. Cultures made from the necrotic lung foci both on horse serum and blood agar developed the characteristic appearances of *B. actinoides*. Only one contained also a fine filamentous growth, probably a streptothrix. All of sixteen cultures prepared from kidney tissue remained free from growth.

No. 474b.—Black and white bull calf, born Apr. 21, 1920. June 3. The calf weighed 135 pounds. The condensation water of three horse serum cultures, 4 days old, showing a dense crop of flocculi was withdrawn into a sterile tube, about 4 cc. in all. This was injected into the trachea, and without removing the needle, about 6 cc. of sterile salt solution were injected through it. After the calf had been placed on its feet it coughed some, ejecting a fine spray. Following the injection the temperature rose about 1.5°C. It was normal next day. The calf was killed June 21, 18 days after the inoculation. The autopsy showed normal conditions with the following exceptions. In the abdominal cavity there are

about 50 cc. of a clear, slightly yellowish fluid which coagulates into a jelly-like mass on standing. Between the vocal cords, along the trachea, and at the root of the right supernumerary bronchus are small masses of a semi-opaque, mucoid substance, consisting of polynuclears, alveolar, and endothelial elements embedded in mucus. The lungs show quite generally over all lobes scattering, very small, dark reddish collapsed or pneumonic foci from mere points to 1 mm. in diameter and of various shapes. In the right cephalic lobe near root is a consolidation about 3 cm. in diameter, but more or less squarish and extending through depth of lung tissue (Text-fig. 6, page 457). It feels lumpy. On section the lumps appear as spherical, firm, grayish foci, about 1 cm. in diameter and centrally necrotic. There is congested lung tissue between them. Each focus is made up of a very firm, almost cartilaginous capsule. Within is a viscid, very thick, whitish mass, which in the larger foci contains in it a more grayish, spongy nucleus found to be necrotic lung tissue. The layer around this contains many mononuclear elements whose cytoplasm, in many cells at least, is filled with fine rods. Masses of similar rods are free in the stained film. The necrotic masses are odorless. Cultures made from the necrotic foci on horse serum showed the characteristic flocculi in 3 days. All contained in addition molds with delicate mycelium. Pure subcultures of *B. actinoides* were obtained, however, from surface colonies. Cultures on blood agar were also prepared. Some were pure growths of *B. actinoides*, others contained one or several other types of colonies. At the same time, cultures were prepared from the air-containing, normal lung tissue by introducing into culture tubes small bits of lung tissue. Molds, liquefying colonies, and several other types of colonies appeared in the tubes.

No. 504b.—Black and white female calf, born June 10, 1920. Weight, June 21, 110 pounds. On June 28, the condensation water of six serum tubes of *B. actinoides* from Calf 474b, 3 days old, was brought together in a sterile tube. The heavily clouded fluid, 5 cc. in all, containing flocculi was injected into the trachea at 3 p.m. The temperature rose about 1.5°C. within 4 hours, then slowly subsided. No abnormal conditions appeared, and it was killed 23 days after inoculation. At this time it weighed 136½ pounds. No lesions were found in the respiratory tract or elsewhere.

No. 520.—Guernsey calf, male. Born Oct. 8, 1920. Weight on Oct. 22, 83½ pounds. On Oct. 27, four horse serum tubes inoculated from cultures of Calf 474b, 4 days ago, were used for intratracheal injection. The condensation water was drawn up into a sterile tube, a little salt solution being used to wash it out more thoroughly. 5 cc. of a moderately clouded fluid were collected in this way. This was injected into the trachea at 10.30 a.m. Before removing the needle, 10 cc. of sterile Ringer's solution were injected. The temperature rose from an average of 38.6° to 41°C. 7½ hours after the injection and gradually fell during the night—the temperature being taken every 2 hours—to 38.8°C. next morning. Following the injection the calf began to appear depressed, the respirations were slightly irregular, about 90 at 2.30 p.m. Attendant noticed a slight chill between 4.30 and 5.30 p.m. The temperature did not go above normal again and the

calf, showing no signs of any disturbance thereafter, was killed Nov. 12. The weight on this day was 96 pounds. The organs were normal with the following exceptions.

Beginning about 5 cm. below lower margin of the larynx, the mucosa covering six intercartilaginous spaces of the trachea is dark red over each space. There are no signs of swelling. In the left caudal lobe a firm mass is buried within air-containing, slightly emphysematous lung tissue (Text-fig. 5, page 457). It is about 1 cm. in diameter. Soft, smooth, flour-paste-like material oozes out from a slight incision. The mass is placed in Zenker's fluid. A film of this thick mass shows necrotic cells and no bacteria. There is a second 2 to 3 mm., firm, yellowish white focus near the first. It contains a nucleus of necrotic lung tissue, enveloped in the same material found in the larger focus. Films of this contain large numbers of minute bacilli among cells of endothelial and leucocyte type which hold the stain as if still living Cultures were not prepared from this case, owing to the scanty material and the need for histological examination of the lesions. This did not, however, show more than has been given from the other cases in which intratracheal injection of *B. actinoides* produced focal necrosis. The central nucleus of the necrotic tissue was surrounded by a layer of polynuclear leucocytes and outside this a connective tissue capsule was forming. Between these two layers, within a narrow zone, colonies of minute bacilli were abundant. Many groups of bacilli were within the cytoplasm of cells.

The results of the inoculation of cultures of *Bacillus actinoides* may be briefly summarized. The subcutaneous injection leads promptly to a large swelling which becomes very firm. After several weeks the mass of the swelling becomes necrotic, the contents caseous, and a thick, firm wall forms with hyperplasia of the connective tissue in the immediate environment of the focus. Softening of the capsule, ulceration of the overlying skin, and discharge outward take place within 4 weeks. In several cases, in which the injections were into the trachea, a small amount of the culture fluid entering, accidentally, the tissues overlying the trachea led to large abscesses. The swelling is primarily due to an increase of mononuclear, endothelial-like elements, with later invasion of polynuclears. The final product is a soft mass, like cottage cheese in appearance, consisting of small lumps, the whole embedded in a thick mass, smooth, glistening, like flour-paste in consistency. In one abscess the remnant of a vessel, 4 inches long, was embedded in the mass. Clouds of minute bacilli are found in the zone outside the necrotic mass where the cellular elements are still stainable. In one case (No. 479b) a second (metastatic) abscess developed at some distance from the site of inoculation.

Injection into the circulation in one case failed to produce any lesions. The second case was sold in excellent condition, probably without lesions. Injection into the trachea failed in two out of five cases to produce any appreciable changes in the lungs. In the remainder there developed small necrotic foci, single or multiple, identical both macroscopically and microscopically with those occurring in spontaneous cases.

The distribution of the induced lesions did not agree entirely with that of spontaneous cases, thus meeting in part the possible objection that the lesions might have been due to naturally acquired infection. The difference in localization was probably due to the fact that the calves were lying on their backs when the culture fluid was injected. This abnormal position may have led to a different drainage of the injected fluid from that occurring in spontaneous cases.

In one instance (No. 495b) the lung lesions were associated with multiple focal scleroses of the kidney cortex.

The culture used was obtained from Calf 462 and passed in succession through Nos. 467, 479b, 495b, 474b, and 520. This was done with the expectation that the virulence would maintain itself. From every one of the above, excepting No. 520, pure cultures were recovered. None were made from the last case.

It will be noted that the intratracheal inoculations produced what was called above the first stage of the disease only. The lesions were restricted in extent and the secondary lesions due to dissemination of the bacilli from the primary necrotic foci did not take place. Perhaps some special depressing conditions may be needed to continue the disease into the clinical stage.

In contrast to the very acute, both destructive and tissue-stimulating action of *Bacillus actinoides* in the subcutis of calves is the following negative action on one sheep.

No. 164.—Barren ewe. On May 11, 1920, the same suspension of cultures from Calf 479b used on No. 495b was injected under the skin in front of left shoulder. Neither local nor thermic reaction followed. The experiment was closed June 1.

Correlation of the Pathological and Bacteriological Data.

The foregoing observations and experiments demonstrate the existence of a specific bronchopneumonia causing mortality in calves chiefly in the 2nd and 3rd months of life. In animals which die within the 1st month other agencies due to fetal conditions and to infections acquired during or soon after birth may involve the lungs. Surviving cases affected with a chronic pathological condition of one or more lobes, characterized by purulent bronchial exudation, abscess formation, and fibrosis may be met in the 4th to the 6th month or even later. As a rule, the disease invades both lungs symmetrically. The parts affected first are the smaller cephalic and ventral lobes, more particularly the dependent portions (Text-figs. 1 to 4). With the progress of the disease the involvement moves upwards towards the dorsal border of these lobes and backwards into the azygous lobe and the caudal lobes. Death takes place when one-half of the latter have become airless. The pleura is involved only where necroses extend to the surface. Here adhesions to surrounding structures form from the capsules of the resulting abscesses.

Several kinds of lesions are presented in the ordinary acute case. The distal two-thirds of the cephalic and ventral lobes are, as a rule, considerably enlarged beyond the normal collapsed state, very firm, dark or light reddish in color. Regularly sprinkled in this ground are grayish, 1 to 2 mm. areas. The proximal third of the same lobes and the affected regions of the caudal lobes are but little enlarged, uniformly dark red, less firm, and without the grayish mottling. Careful search, by manipulation if necessary, reveals scattering firm masses, sometimes deeply embedded, more commonly extending to one or both lung surfaces. They vary from 2 to 10 mm. in diameter. Several may coalesce. They consist of a pearly, dense capsule containing necrotic lung tissue, enveloped in a layer of viscid pus. These sequestra are with rare exceptions located in the cephalic and ventral lobes. They are probably among the oldest lesions. They and the mottled pneumonic regions which are always associated with them are either developed at the same time or else the necroses are the source of the infection which produces the pneumonic condition. The smooth dark red pneumonia is secondary to the other lesions,

probably through the agency of aspirated purulent exudates. The distribution and extent of the three conditions are shown in the text-figures. The pneumonic condition is usually not universal in the affected lobes. Small or large air-containing territories may occur in them, chiefly along the free margins of the lobes. The one universal characteristic lesion is the filling of the air tubes of the affected lobes with a thick, viscid, glairy, white, mucopurulent matter.

The microscopic characters of the different stages are fairly well definable. The formation of the necroses (Figs. 12, 15, and 16) has not been traced, owing to lack of material in the early stages. Associated with or following these, there is a filling up of the alveoli with several cell types to form the mottled pneumonic territories. At the same time the ultimate bronchioles and alveolar ducts may become involved. The entire parenchyma becomes filled with mononuclear elements, probably a mixture of alveolar cells, endothelial and lymphoid cells. The polynuclear leucocyte is absent in this stage. The smooth, fresher pneumonic condition which develops later is due to partial or complete filling up of alveoli and bronchioles with polynuclear leucocytes. In these the epithelium remains intact. With this stage there appears the zone of plasma cells around the air tubes filled with cell debris (Figs. 7 and 13). Numerous polynuclear leucocytes are found moving outward through the epithelium into the lumina. This accumulation of plasma cells is probably due to the cell debris in the lumina of the air tubes. They are present after a certain time whether one or several species of bacteria are at work. That they are the result of the stimulus exerted by toxins and other products absorbed from the disintegrating cellular plugs in the lumina seems to be at present the most plausible explanation of their presence. The minute grayish dots permeating the older pneumonic lesions, which are so striking in the fresh lung of most but not all cases, have not been associated definitely with any microscopic details. They may represent the earlier proliferative lesions in the ultimate bronchioles in some cases and the gathering of plasma cells in later stages.

Before summarizing the results of the bacteriological studies it should be stated what is apt to be forgotten in an interpretation of results, that the normal lungs of calves, and other species as well,

contain a considerable variety of living microorganisms. When bits of normal lung tissue are placed in culture tubes, growth appears quite invariably. Spore-bearing bacilli, various kinds of molds, and streptothrix are among the commonest forms detected. In the isolation of *Bacillus actinoides*, on agar, bits of lung tissue are essential to growth. On coagulated serum tissue is not necessary. It is somewhat surprising that so many cultures obtained from bits of diseased lung tissue were pure cultures of *Bacillus actinoides*. It would seem as if under the influence of the violent tissue reaction the banal forms found in normal lungs are largely destroyed.

The bacteriological examination has shown a variety of results, depending on the stage of the pneumonic process, the rapidity of its development, and whether the animal died or was killed. In the 1919 to 1920 epidemic, twelve cases were investigated. *Bacillus actinoides* was the only cultivable organism present in five of these. It was present but associated with other pathogenic species in four more. It was not detected in the remaining three. *Bacillus pyogenes* was present in seven cases but not in pure culture. *Bacillus bovisepticus* was present in three or four cases, always with a variety of other species. Of the five cases in which *Bacillus actinoides* was exclusively present, two had died and three were killed. Of the four in which it was associated with other species, three were killed and one died. Of the three in which it was missed, two died and one was killed. A study of the brief protocols suggests that *Bacillus actinoides* was missed through overgrowth with other species in certain very acute dead cases and in the more chronic surviving cases.

In the tissues of the diseased lungs *Bacillus actinoides* is found in the peripheral layer of lung sequestra, in colonies in the cell masses occupying alveoli and alveolar ducts (Figs. 8 to 11), and in the proliferated epithelium obstructing the bronchioles less than 0.1 mm. in diameter (Figs. 1 to 7). This latter process which is rather unique and which has not thus far been described in the pathology of pneumonias was especially well brought out in sections of No. 440, but it could be detected in most other cases after more or less searching. In the invaded epithelium *Bacillus actinoides* appeared with the sheath or capsule around it. In other situations it was usually free from capsules and appeared in dense, cloud-like colonies. It should

be stated here that, with rare exceptions, air tubes over 0.1 mm. in diameter had their epithelium intact. The cell masses with which many were filled—the glairy, mucopurulent contents—consisted of cell debris moving up from the ultimate bronchioles and the parenchyma.

After injection of pure cultures into the trachea *Bacillus actinoides* has thus far produced only the early stage of necrosis (Text-figs. 5 to 7 and Figs. 15 and 16). The diffuse secondary and later lesions characterizing the spontaneous fatal and very sick cases killed were absent. The relation of *Bacillus actinoides* to the primary necrotic lesions is thus placed beyond doubt. That it is also responsible for the general pneumonic involvement is not proved by experiment but made highly probable. Neither *Bacillus pyogenes* nor *Bacillus bovisepticus* is responsible, since these easily cultivated bacteria were absent in five advanced cases. Considerations based on bacteriological and histological studies are sufficient to throw them out of the other cases in which they were found, except in the rôle of continuing a disease fully under way by multiplying in the necrotic tissues and the bronchial cell debris. If *Bacillus pyogenes* and *Bacillus bovisepticus* are not responsible for the diffuse pneumonic lesions, then either *Bacillus actinoides* or else some non-cultivable, unrecognized microorganism is. The writer has throughout assumed that *Bacillus actinoides* is the sole responsible agent.

A study of the mode of response or reaction of the tissues to *Bacillus actinoides* has not come within the scope of the present investigation. The finding of some small susceptible species is necessary to provide material for such study. In general the reaction in the lungs is at first associated with mononuclear cell types, either endothelial or lymphoid or both. In later stages polynuclear cells completely dominate the process. This change may be due to an immunity reaction. Many things point to this explanation. Thus the later smooth pneumonic condition is always associated with the polynuclear cell type. In those animals in which the process halts with the necroses, the remaining parenchyma contains only polynuclears in focal distribution. Moreover, *Bacillus actinoides* is only very rarely detected with the microscope in the lesions associated with polynuclear cells, although cultures reveal its presence. This rarity suggests that the active stage of multiplication is over.

The various factors entering into the process such as quantity and dissemination of the original infecting material, relative susceptibility of the host, and the rapidity with which immune forces are called forth determine the extent and rapidity of the early, usually necrotic changes and the promptness of dissemination of the virus from such necrotic foci over new territories. Added to these factors are species of bacteria, chiefly *Bacillus pyogenes* and *Bacillus bovisepticus*, which may graft themselves on the diseased tissues. In thus restricting *Bacillus bovisepticus* to a secondary position, the author does not imply that there may not be virulent races of *Bacillus bovisepticus* capable of initiating outbreaks of pneumonia.

Aside from Case 6, no detailed description of the cases occurring in the 1917 outbreak, which was the basis of an earlier publication,[1] has been given. This group of cases had not been worked up with so much care and in such detail partly because the very first case which came to autopsy yielded pure cultures of *Bacillus bovisepticus* and the developing epizootic was considered due to this bacillus. With the succession of cases, the appearance of a polymorphic bacillus and its association with a closely resembling species, *Bacillus pyogenes*, rendered orientation very difficult until a pure culture of *Bacillus actinoides* was made to grow indefinitely in subcultures and its forms clearly differentiated from *Bacillus pyogenes*. A repeated study of the accumulated data has shown such a parallelism between the 1917 and the 1919 cases in gross and minute anatomy and histology of the affected lungs and in the bacteriology that any review of these early cases would be in the main a repetition of what precedes. The underlying etiological factor and the processes it initiates are the same in both groups of cases.

A study of the epidemiology of the pneumonia due to *Bacillus actinoides* needs much additional material. The source of this microorganism is not yet defined. It will, however, be safe to regard partly recovered older animals of the same species as the purveyors of the virus. The termination of certain lesions in necrosis followed by copious discharge from the resulting abscesses opens the way for the virus outward. It is probable that unrecognized cases occur in which the process does not go beyond localized necrosis of small territories. Such cases may serve in maintaining the mild disease

until the cold season favors the secondary extension of the lesions and brings acute clinical cases to the surface. This view is supported by the production of necroses experimentally in calves without any appreciable rise in temperature or other suspicious deviations from health.

Owing to the meager descriptions given in reports of outbreaks of pneumonia in calves, it has been impossible to identify this form of bronchopneumonia in earlier writings. An attempt was made in the article on *Bacillus actinoides*[1] to correlate the latter with Lignières' actino-bacillus which produces subcutaneous and other abscesses but not pneumonia. A continued study of *Bacillus actinoides*, however, does not make the relationship seem any more real. It remains to study calf pneumonias in both anatomical and microbiological directions afresh, since the existence of several etiological types of lung disease is probable. The anatomical and histological study of these pneumonias is important, since information gained in this way tends to restrain hasty conclusions concerning the etiology where several different species of bacteria may find opportunity for multiplying in the lungs in the course of the disease.

CONCLUSIONS.

A bronchopneumonia of calves in the early months of life is described and its etiology associated definitely with a minute bacillus, *Bacillus actinoides*. *Bacillus pyogenes*, *Bacillus bovisepticus*, and, less frequently, staphylococci and streptococci may appear later in the affected lungs.

Subcutaneous injections of cultures of *Bacillus actinoides* produce large indurations ending in necrosis. Similarly intratracheal injections produce circumscribed necroses of lung tissue. The cultivation of *Bacillus actinoides* and its morphological peculiarities have been sufficiently described and illustrated in an earlier publication[1] to ensure success on the part of those who attempt to isolate it.

The writer is indebted to Dr. R. B. Little for assistance in bringing together the clinical data.

EXPLANATION OF PLATES.

PLATE 42.

FIG. 1. Section of lung tissue, Calf 440, showing dilatation of a small bronchus and complete filling up of the lumen with the characteristic exudate. The epithelium has disappeared from most of the circumference. The exudate is intimately associated with the subepithelial tissues. The parenchyma around the bronchus is partly collapsed, partly pneumonic. The capillaries are distended with corpuscles, and the alveoli are filled with cells of endothelial and lymphoid type. See Fig. 4 for magnification of a part of this exudate. × 240.

PLATE 43.

Both figures from Calf 440.

FIG. 2. An air tube about 0.1 mm. in diameter with ingrowth of cellular masses, which are filled with bacilli, as shown in Fig. 5. The section passes through a dividing bronchus, of which one branch is involved. Remnants of the epithelial cells are traceable in the irrupted mass which contains cells with round, pycnotic nuclei. × 240.

FIG. 3. Transection of an air tube about 0.3 mm. in diameter. The exudate is intimately connected with the subepithelial tissue of the bronchus in two places. Elsewhere the epithelium is still *in situ*. The exudate is of the same character as in Figs. 1 and 2. It is permeated with cloud-like masses of minute bacilli showing only as slightly darker patches in the photograph. × 310.

PLATE 44.

FIG. 4. A portion of the diseased bronchus of Fig. 1, enlarged × 1,000. The bacillar and capsulated or club-shaped forms are present, filling the cytoplasm of the cell mass which has nearly plugged the lumen of the small bronchus.

FIG. 5. A portion of the diseased bronchus of Fig. 2, enlarged × 1,000. The bacillar, cloud-like masses have filled the cytoplasm of the cells as in the preceding figure. The club-shaped forms are shown in the lower right-hand region in many cross-sections and rather sparsely elsewhere in the bacterial masses.

PLATE 45.

FIG. 6. From Calf 440. Section through a small bronchus, of which the epithelium along one margin (to the right in the figure) is still *in situ*. The other margin is obliterated by an ingrowth which nearly occludes the lumen. This ingrowth is densely permeated with *B. actinoides*. The bacilli appear chiefly as very short rods of irregular size and form throughout the section not taken up by the bronchial epithelium. × 1,000.

FIG. 7. From the same lung. The small air tube is nearly occluded by the mass of cells on the left. This mass is permeated densely with *B. actinoides* in bacillar form. In addition, larger circular areas, suggesting capsulated cocci, are sprinkled through the mass. These are identified as club-shaped forms of *B. actinoides* cut across. × 1,000.

PLATE 46.

FIG. 8. Section through the parenchyma of the lungs, Calf 440. The alveolus is filled with cells. In the center of the cell mass is a colony, or floccule, of *B. actinoides*. × 1,000.

FIG. 9. Two alveoli, each containing a floccule. The clubs are shown imperfectly in optical cross-section. × 1,000.

PLATE 47.

FIG. 10. Section from an old case, designated as Calf B on page 454 of the text showing two foci in which are cloud-like colonies of minute bacilli, appearing as irregular darker patches in the figure. × 1,000.

FIG. 11. Section from the lungs of Calf 440. In the upper left-hand corner is a large cloud-like colony of bacilli. Other colonies in the field are out of focus and appear as slightly darker patches. × 1,000.

PLATE 48.

FIG. 12. Sequestrum from the lung of Calf 462, slightly dislodged. The outlines of the alveoli may still be distinguished in the upper portion of the necrotic mass. The border of the wall or capsule above, which suggests epithelium in the figure, is compressed tissue made up of fibroblasts and plasma cells. × 25.

PLATE 49.

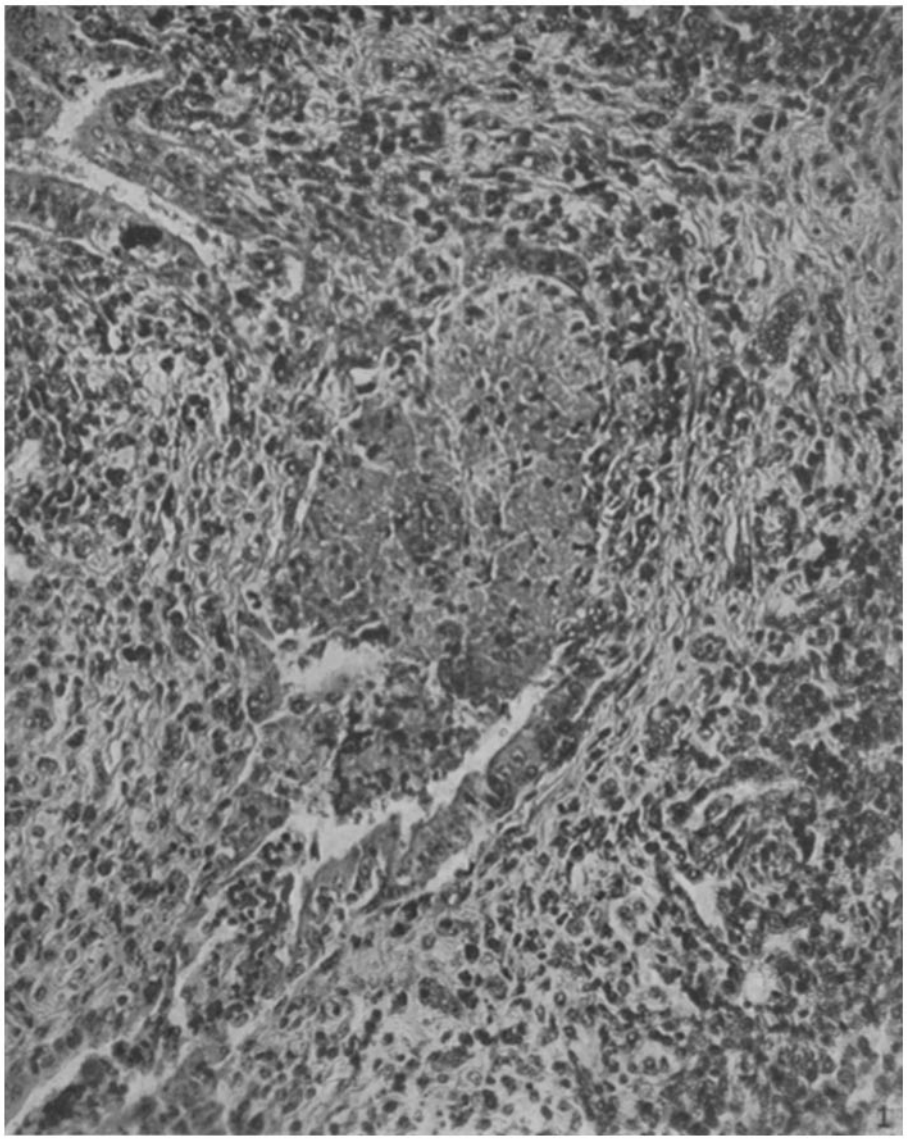
FIG. 13. Section from the lungs of No. 6. The more or less deformed bronchi are filled with polynuclear leucocytes and surrounded by a broad zone of plasma cells and fibroblasts. × 925.

FIG. 14. Section of lung tissue from Calf 446. This case is somewhat older than No. 440. Near the center of the figure is a more or less deformed bronchiole containing polynuclear leucocytes. The lung tissue in a broad zone around this tube is occupied by groups and masses of plasma cells and polynuclear leucocytes. × 925.

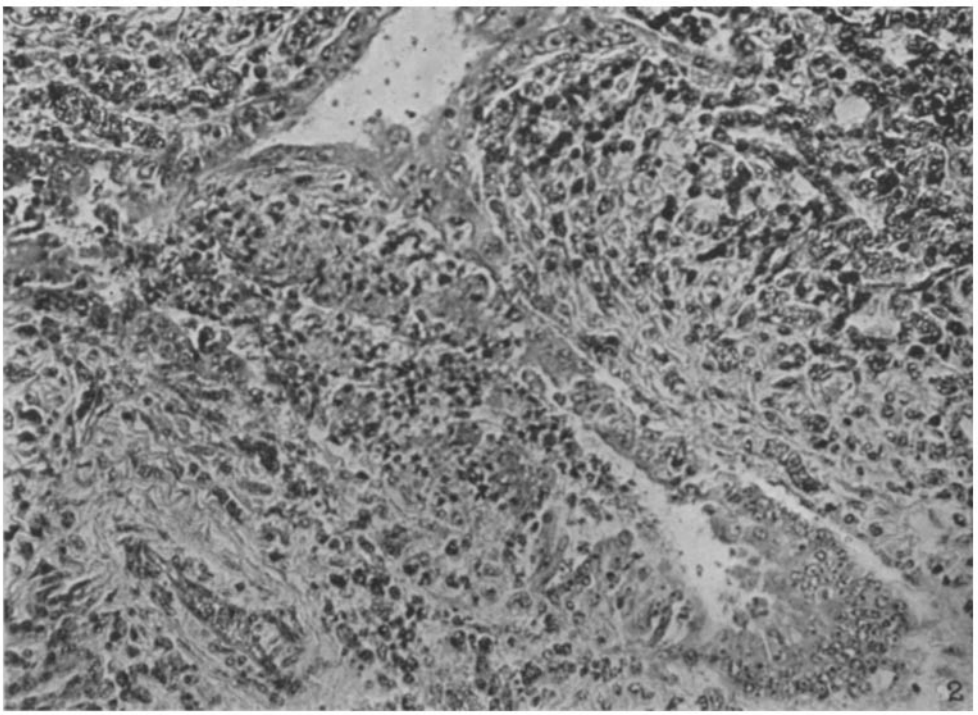
PLATE 50.

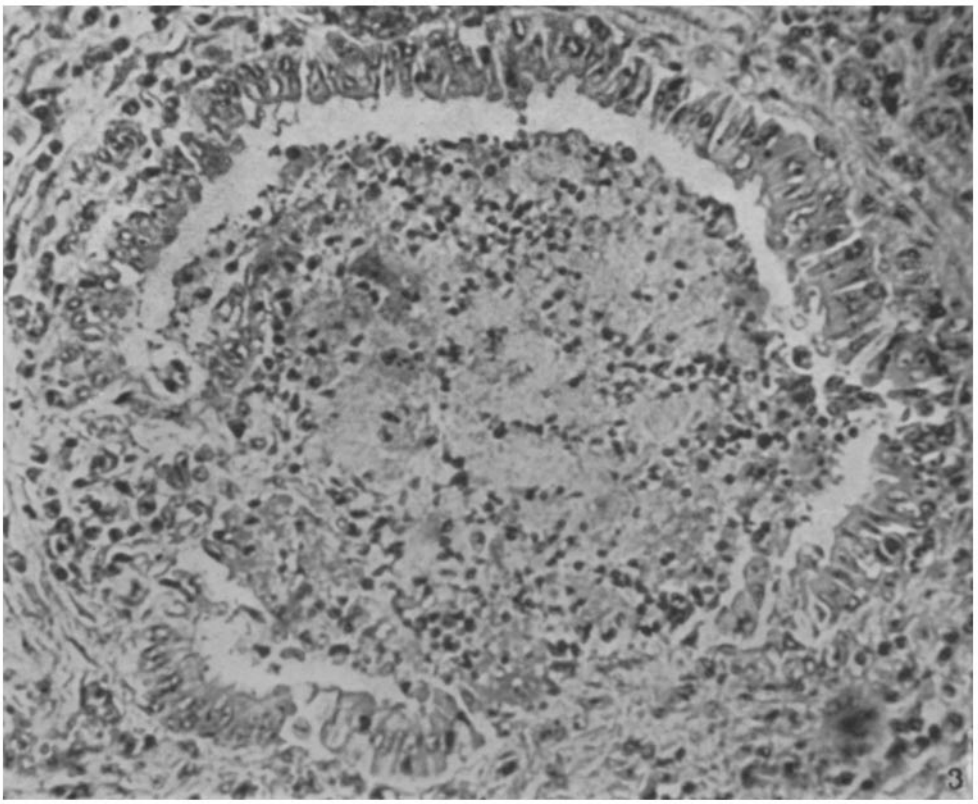
FIG. 15. Represents a necrotic focus from the lungs of Calf 495b (see Text-fig. 7) which received an intratracheal injection of *B. actinoides*. × 60.

FIG. 16. Enlargement of the zone just outside the necrotic mass shown in Fig. 15. Irregular groups of *B. actinoides* occupy this zone. The large group on the right has only a few bacilli in focus, whereas the central group is fairly distinct. × 1,000.

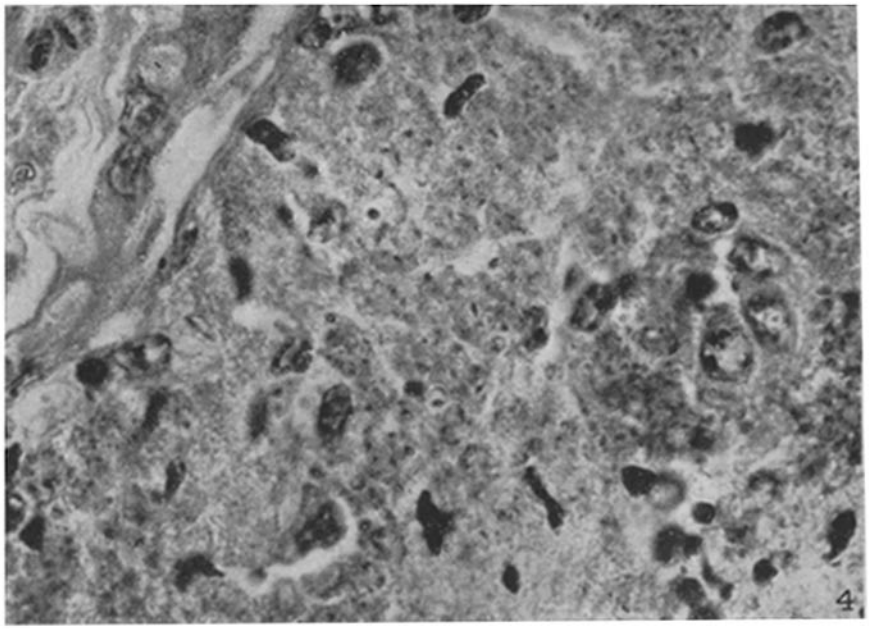


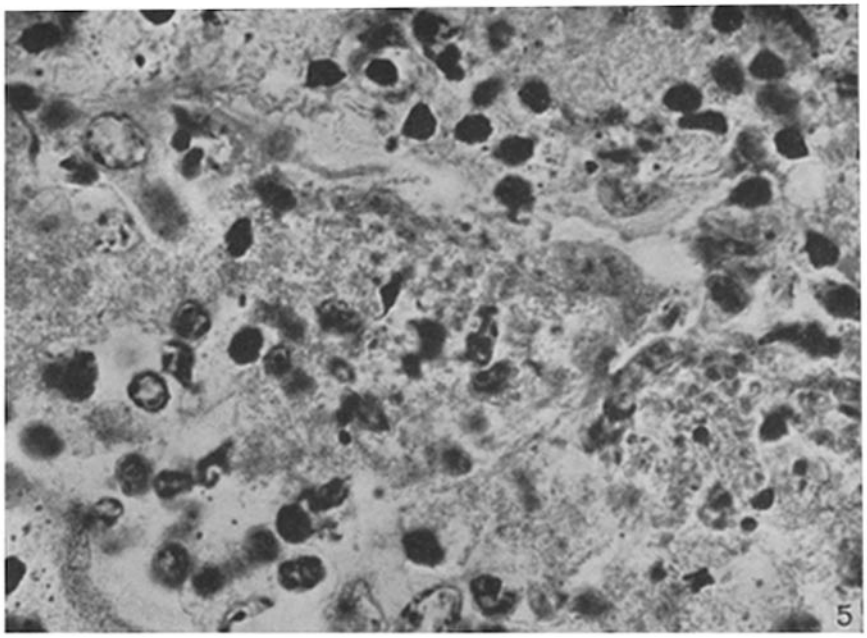
(Smith: *Bacillus actinoides.*)





(Smith: *Bacillus actinoides.*)





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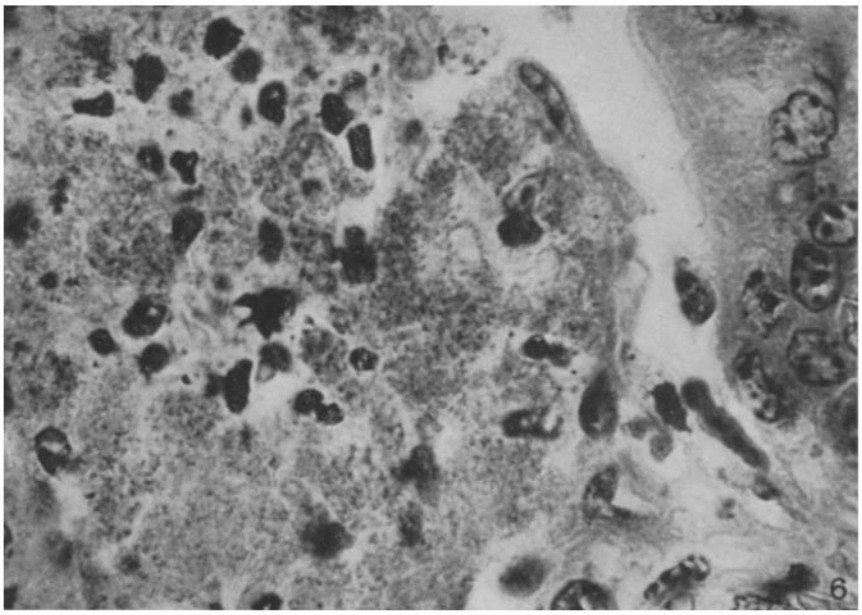
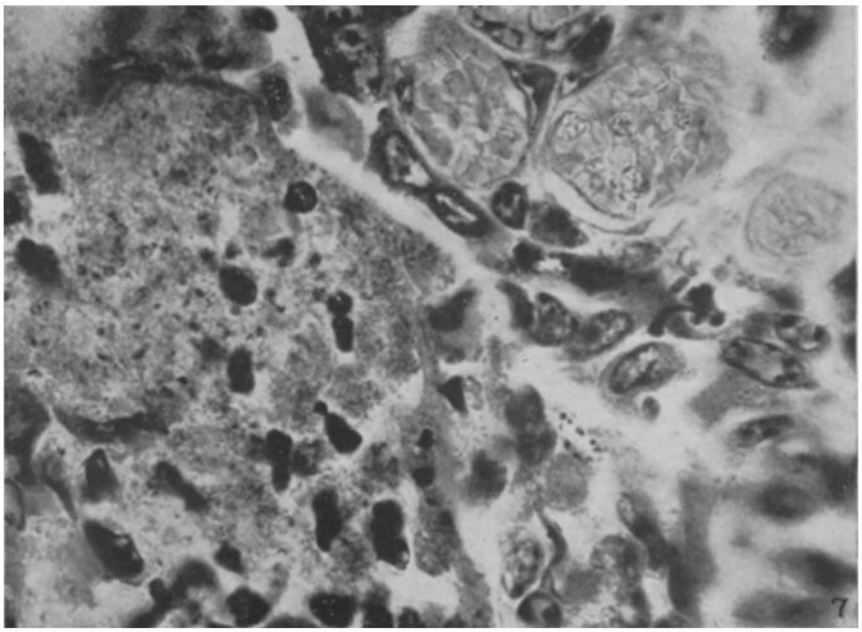
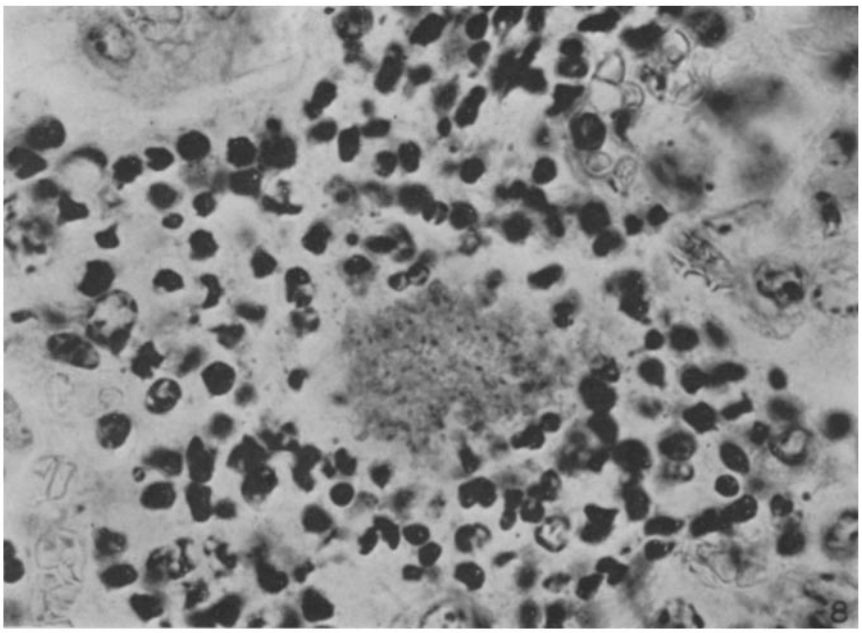
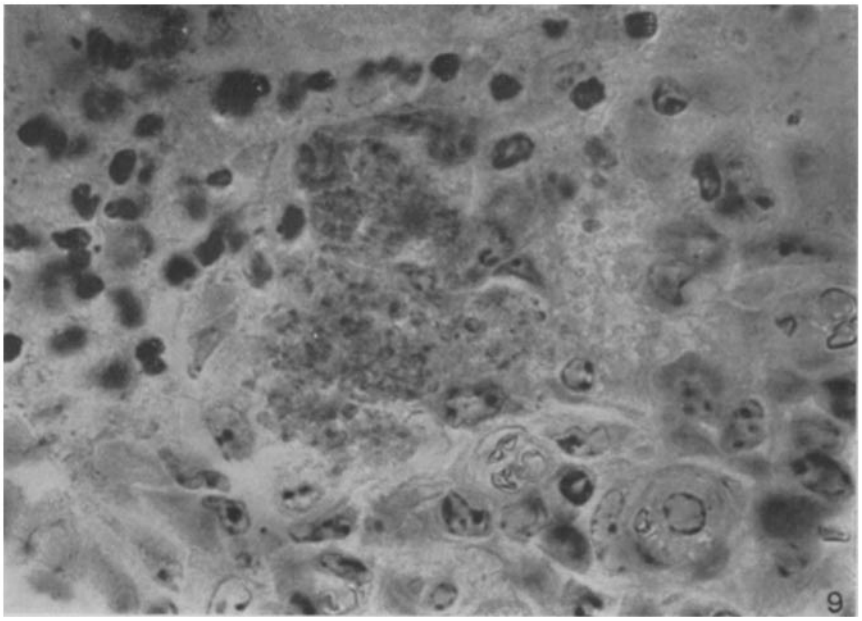


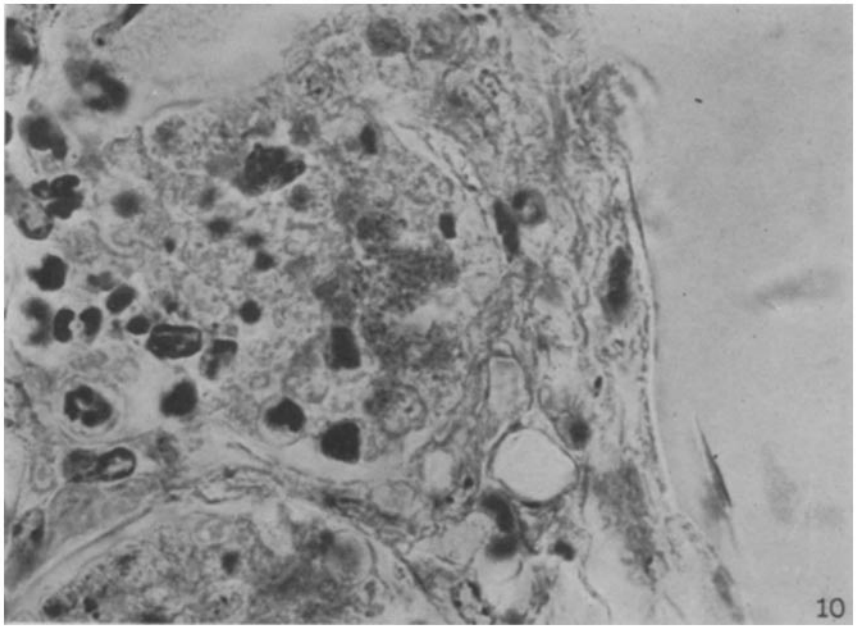


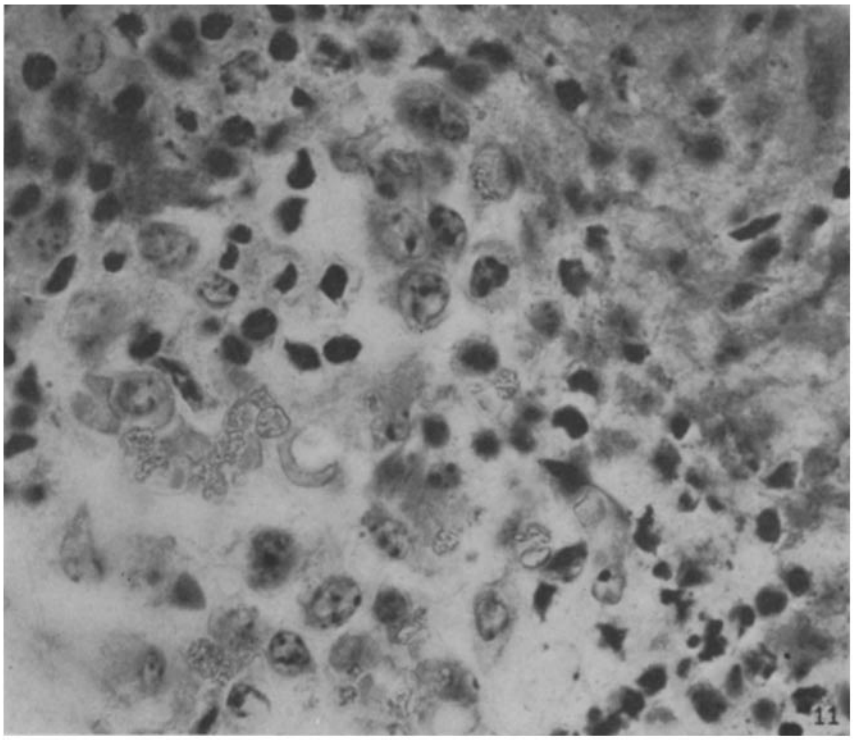
(Smith: *Bacillus actinoides.*)



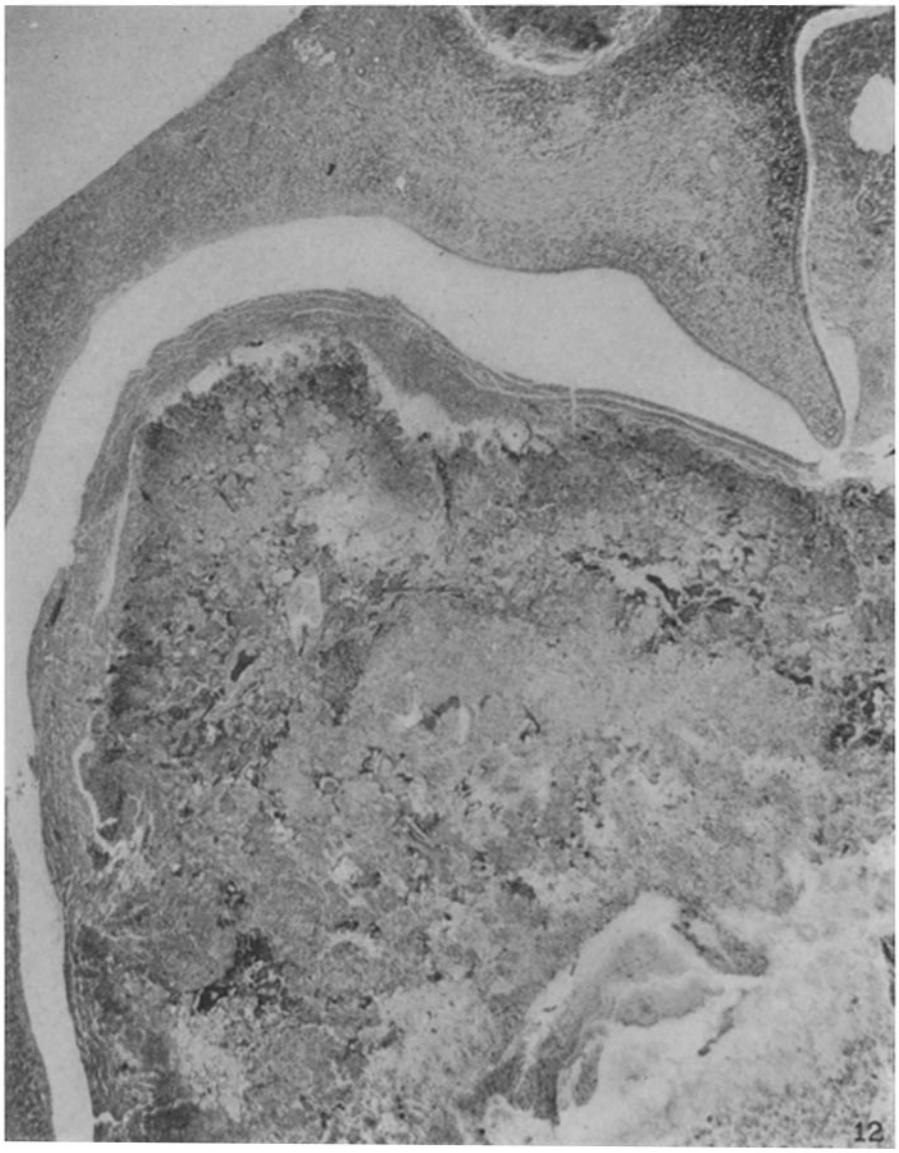


(Smith: *Bacillus actinoides.*)

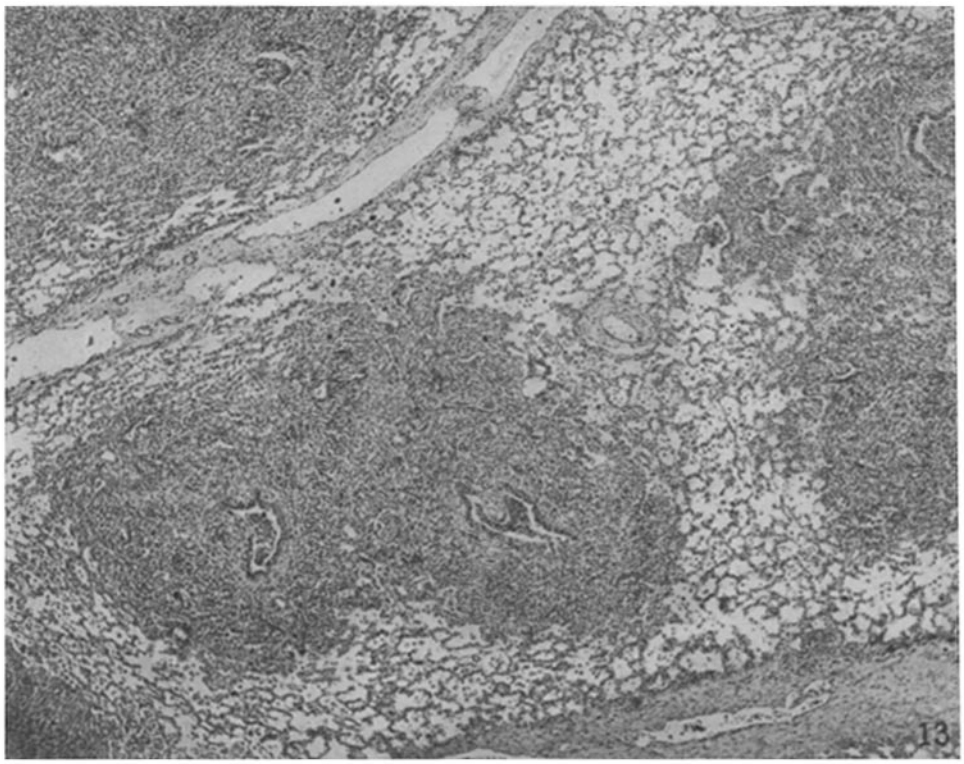


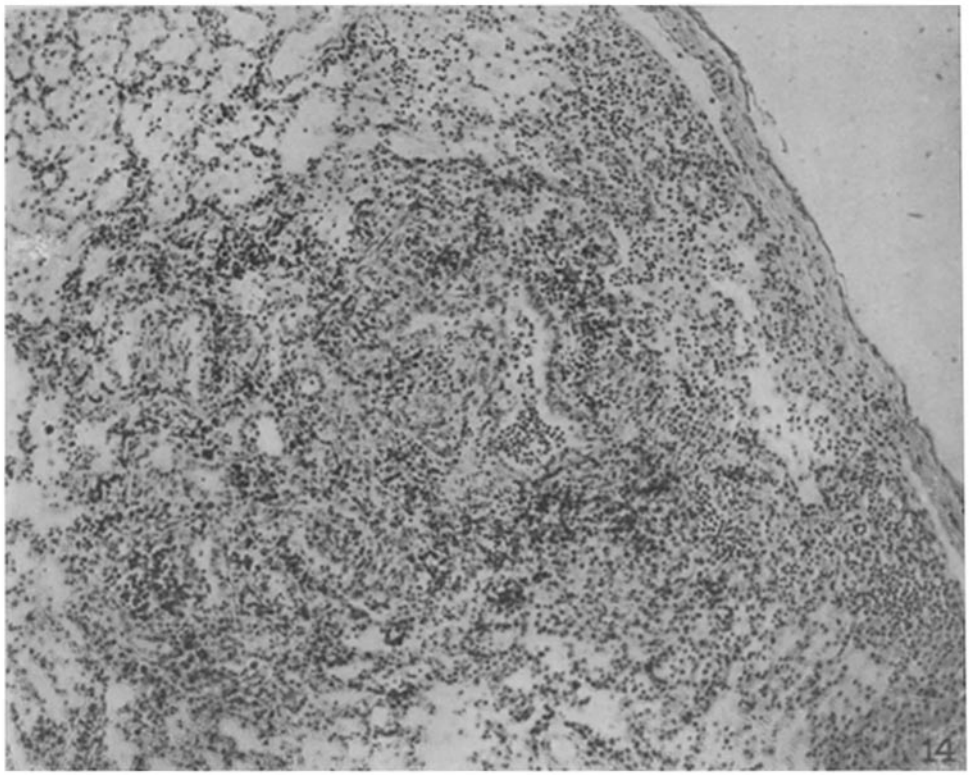


(Smith: *Bacillus actinoides.*)

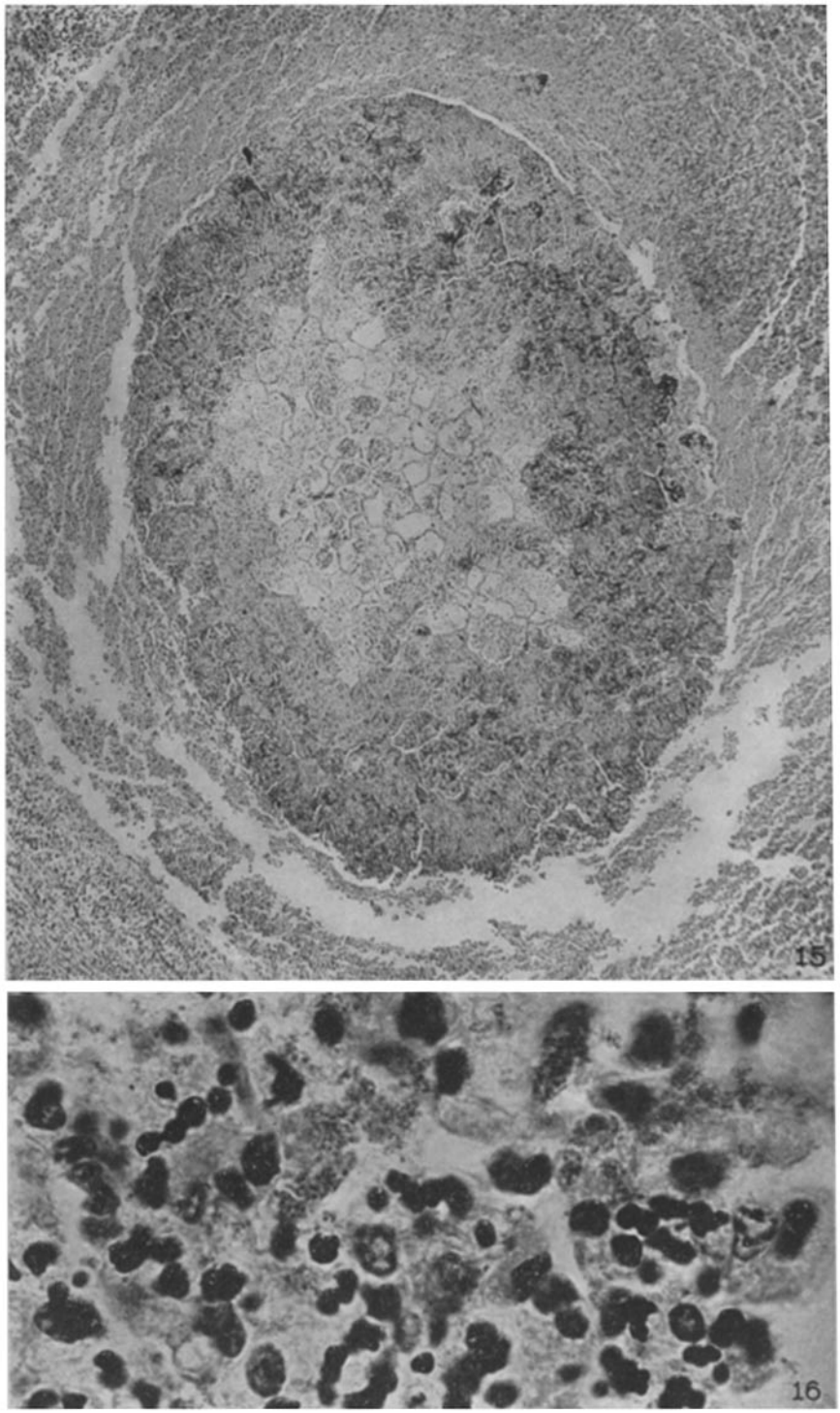


(Smith: *Bacillus actinoides.*)





(Smith: *Bacillus actinoides.*)



(Smith: *Bacillus actinoides.*)